Hints on Collecting and Growing Algae for Class Work.

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A great deal of worry and trouble is usually anticipated by the teacher in obtaining cryptogamic material for classes of botany. During the past few years I have received so many requests from high school instructors regarding methods of procuring such plants, that I thought it advisable to outline briefly some general and particular methods that may be of help to the teacher. Necessarily many of the hints here given will be well known to the algologist, but the average instructor in botanical technique may not have had the time and opportunity to acquire experience which to the expert is likely to seem commonplace. Many, apparently even experienced instructors, well acquainted with the higher plants, I have found confess that they are unable to obtain the most common and ordinary forms of the lower plants.

As a matter of fact it is not especially difficult to obtain or even grow the lower plants, and most of them once gotten are easier to keep a long time than the phanerogams. There is required, of course, a certain knowledge of the habits and methods under which the development of algae proceeds best, but with a little experience, acquired only by patient reiterated efforts at growing such, their response to attempts at cultivation will soon be surprisingly gratifying.

Our common text-books and even systematic treatises do not give us much help in growing such plants, and for the most part do not even state clearly the habitat of the same. Nothing is said about the cultivation of lower plants in the laboratory. The object of this article, then, is to supply in a limited way, some hints for obtaining and growing the common types of algae, from the field or laboratory aquarium.

Here, more than in any other undertaking experience, often fraught with failure is the best instructor and guide, but as it is also true that most teachers have not even the right idea how to make a start, I shall feel repaid if I can hope that some, at least, will realize as the result of these few hints that it is worth while to make the attempt even though many failures result. A few suc-

cesses will repay for many failures. At the very outset we shall find that growing algae in the laboratory will be, on the whole, an easy matter in spite of the fact that at present we know very little about the subtle influences to which these plants respond. We may often feel obliged to refer our successes to luck, but as the chances of success and failure are about equal on the average, the results of the game are worth the venture, and after a number of failures we come to feel that a series of successes are due to us as a result of effort, while he that never tries dares not even hope to succeed even once.

Nearly all the plants ordinarily demanded by the common text as types for instruction can, with reasonable foresight be obtained in the laboratory aquarium by development, or be found out of doors in nearby ditches and pools. Often preserved material answers our demands almost as well as live material, so that if any particularly good specimens are found in the field, they should be gathered and "pickled" in as large a quantity as obtainable. Though plants will often appear annually or periodically in places where once found, it is well to consider that the next periodical appearance in abundance in any given locality may be as long as ten years away, and that it is therefore best to obtain as much as possible even at the cost of considerable inconvenience.

We know little or nothing about the periodicity of the reappearance of algae in any given place, and hence there is no other circumstance when it is so much worth while to make the best of an opportunity, as in case of an abundant appearance of some rare specimen of algae.

I shall first discuss some general considerations helpful to success in growing algae in the laboratory, and then give detailed methods for some common individual specimens.

The first and most common mistake made by those that attempt to keep algae alive in the laboratory is to change the water in which these plants are. The motive for so doing is a well intended one, but there is a fallacy connected with it. The reason usually given is that a change of water prevents decay by the action of bacteria. Paradoxical as it may seem there are few if any of the hardiest algae that can stand such hard treatment though well meant.

A little reflection will show that since it is so difficult for the lower plants to accomodate themselves to their environment, a

daily change of water means a daily change of environment, and they have no sooner made an attempt to react favorably to their surroundings and begun to accomodate themselves when new conditions are again forced upon them. Therefore changing more than once the medium in which they are, is doing them the greatest harm possible, and under the circumstances algae almost invariably die, and disappear very soon, sometimes not to return. It is, therefore, just because these plants are so delicate and are affected by influences so subtle that we do not yet fully understand them, that we must refrain from multiplying the difficulties under which they thrive. The fact that most of these plants are normally found in stagnant or still water, which nature does not change, at least during their lifetime or growing period, is proof enough that they grow best in water in the laboratory which most resembles their natural habitat. Some algae like *Chætophora*, *Batrachospermum* and *Ulothrix* are found in running or dripping water in their native abode. Such plants are, however, permanently transplanted from the field to the laboratory with the greatest difficulty. It is almost impossible to preserve live *Ulothrix*, for any length of time, whether put into still or running water after collecting.

In general, plants must be brought from the field to the laboratory with conditions as near as possible to those under which they are found, and so kept. It is always best, when possible, to put into the vessels the very water in which they were obtained. Some algae can not stand the shock of being transferred to other water in the aquaria. A single change of water is fatal to them. Such delicate forms may, however, be obtained and made to grow by a method which will be outlined in the course of this article.

Algae which can withstand the shock of a single change of water from the field to the tap water in the aquaria will present no great difficulties in their successful growth. Often it will be found that they disappear for the time being. The beginner will conclude that they have completely disappeared as the result of bacterial action, or death, when they have only gone into some resting stage in the form of reproductive spores, usually as hypnospores. The temptation then comes to throw out the contents of the jar and begin over again with another culture. Starting another culture is of course a good thing to do, but it is also good to leave the contents of the jar for a few months, and it will be invariably found

that the disappeared forms will rather suddenly come back. often in better condition than when first found in the field, or if the plants do not appear, others equally or more valuable will develop, for which we have sought with as much care. Algae are never found in their native habitat in pure culture. The disappearance of one specimen is often but the reason of the appearance of another that only waited for its predecessor to go in order to come upon the scene itself. When the same algal forms reappear it is but a sign the resting stages of the reproductive organs had been slowly adapting themselves to an environment in which they finally found it possible to germinate and thrive.

The contents of an aquarium need only be thrown out when complete organic decomposition has taken place which is indicated by the presence of bacteria, often by a purple-violet coloration of the water and always by an over-powering disagreeable odor of decay, Even after this, however, desirable plants in form of Myxophyceae especially *Oscillatoria* have subsequently been known to develop. Another instance in which it is advisable to pour out the contents of the jar is when a small form of *Oscillatoria* appears. This is easily recognized under the high power of the microscope, but even as a type of *Oscillatoria* it is not valuable, as it is too small to serve for demonstration. The cells and contents are better brought out by larger forms. Moreover the plant prevents the development of all other desirable plants except other Myxophyceae such as *Glaeocapsa*, *Chroococcus*, *Anabaena*, *Nostoc*, *Tolypothrix*, *Cylindrospermum* and *Rivularia*. Whenever this small *Oscillatoria* obtains possession of a jar it destroys everything else either by crowding it out or choking it to death or actually posioning it, by means of some evil smelling compound partly soluble in the water in which the plant is found. Even *Cladophora* which is one of the most hardy of the algae and which will stand continuous changes of water quite well, and will remain several years unchanged in a small bottle without being affected noticeably, will disappear quickly under the influence of this small dark blue *Oscillatoria*.

Complete removal of this enemy of the aquarium is only effected after very thorough disinfection of vessels with formaldehyde, or another equally energetic disinfectant. The *Oscillatoria* does not seem to prevent the growth or reappearance of other Myxophyceae (Cyanophyceae or Blue Green Algae) and a good culture of any of these need not be disposed of to prevent the ravages of the enemy.

It is, however, best to keep the *Oscillatoria* out of the laboratory as completely as possible, because if tolerated in but one jar it will almost unaccountably spread to all the others until it has become so prevalant that no plant can be raised or kept in the laboratory until the whole room has been thoroughly and completely disinfected. I have found this plant a greater enemy to the laboratory and aquarium than any and all bacteria together. The latter, as a rule, have definite periods of growth and for a long time their ravages are interrupted, but the *Oscillatoria* is always present and never ceases its ravages. Hot water is not sufficient to kill the plant. Some of the congeners of this plant are known not only to exist but actually to thrive in the almost boiling water of some of the springs and geyser basins of Yellowstone Park.

Bacterial decomposition can best be prevented by avoiding the introduction into the jar of too much of the collected specimen. But a small amount of any given specimen should be put into a jar, about a cubic inch of plant mass to the gallon. Nature will often subsequently grow plants in larger proportion both in laboratory and the field than this, but nature has also already gradually accommodated the plants to the environment, and this accommodation has not been effected when we start a new culture.

It is advisable to have small aquaria. The jars should not ordinarily be larger than six gallons capacity and the best results have been found to take place in one or two gallon vessels. Aquaria of twenty gallon capacity are good for raising crustacea entomostraca, worms, insects and even higher animals but these are among the greatest enemies to the algae. *Cladophora* also develops beyond all bounds in large vessels once it has gained access, and usually spreads so rapidly as to choke out more desirable plants.

Large insects in an aquarium should be removed. No good ever comes of their presence, and many destroy plant life. Water snails are very destructive and reproduce very rapidly. Good crops of desirable plants will result after *Branchypus*, or water shrimps have disappeared, but their presence in a jar of plants is not desirable, and with the organic matter which they consume they certainly consume a considerable amount of unicellular algae and reproductive stages of higher algae. Entomostraca and small crustacea are great enemies to growth of plants and it is very difficult to get rid of them because of their small size.

A good check can be put upon them, however, by introducing

temporarily into a jar some *Utricularia*. This plant has its leaves provided with little bladders in which entomostraca and small water insects are caught in large numbers and removed from the scene of their ravages. These little bladders are very numerous and I have found at times as many as half a dozen small insects captured in a single one.

The jars in which filamentary algae such as *Spirogyra*, *Mougeotia*, *Zygnema*, *Œdogonium* and others are grown give better results when the bottom of the vessel is covered with about an inch of clear washed sea-sand. In some manner not quite evident, I have found that jars with sand in them are not so likely to decay by bacteria. To clean the sand it is sufficient to allow a stream of hot water to drip on it in the jar for half an hour. If the sand had ever been present where *Oscillatoria* or other Myxophyceae had been it should be disinfected by soaking over night in formaldehyde and subsequent washing with cold and hot water in turn.

The water which is used in the aquaria should be ordinary pump or tap water,—the former being preferable. If tap water is used it should be allowed to run quite a while, at least five or ten minutes before the jar is filled. The water standing in pipes takes up, especially in presence of carbon dioxide, small and often inappreciable quantities of iron, lead, or other metals, in solution, probably not from the metal, but from the ever present oxides and rust. These minute quantities of metallic salts are very destructive especially to the conjugatae and unicellular plant forms. Hard water, or water with lime in solution is also not the best for the abovementioned plants. The hardness of the water can be almost totally removed after a time by the introduction into the jar of one or several young green plants of *Chara*. This plant has a large percentage of lime in its cell wall and cell contents, and grows best in water when lime is present: and it serves well to eliminate it from the aquaria. This is probably the reason why Chlorophyceae in the presence of *Chara* react more promptly in accommodating themselves to their environment when put in the fresh water of the laboratory jars.

Some of the plants introduced into the laboratory jars from the field will take kindly to their environment if the water is not thereafter changed and the precautions and methods of treatment as outlined are followed. Others will disappear for the time and later come back in greater or less abundance most of them passing easily

into their reproduction stages. A quicker way than this latter more or less uncertain one has been devolved and considerable success attained. It has been found that if mud, grass, sticks of wood and the like are taken from the place in the field where certain plants have been observed or are known to have been present, and this mud is put into the laboratory jars, there will often in the course of a few weeks result a luxuriant growth of the plant even though no traces of the plant can be found at the time of the collecting. This method is especially applicable in winter, and then most striking in its results. The method applied at that season is not unlike that used in "forcing" the higher plants for flowering at Easter. An example which was several times repeated will illustrate this. In the winter of 1907 some mud, wood, and weeds that were found in an old barrel in the ground from which a spring gushed part of the year, were brought into the laboratory and put into a gallon beaker. Nanandrous *Œdogonium* had been collected there in the preceding spring and the material was excellent. The time of collection was about the middle of December in the course of a few warm days when the snow disappeared and the ice had thawed out by the warmth of the sunshine. A day or two after the material was introduced into the laboratory a blizzard came up rather suddenly. Snow fell that did not leave the ground till spring. In the laboratory the jar became covered within a week with a green coating, and it was found to consist of numerous zoospores which soon attached themselves to the walls of the vessel, changing their cilia into "holdfasts." They grew rapidly and in great abundance so that within a month and a half, I had a large amount of the best nanandrous *Œdogonium* I have ever collected. The culture was practically pure, and had the advantage that it could easily be removed from the glass walls of the beaker without injuring the plants in the least. From time to time the plants were removed, and were found in all stages of sexual reproduction. As fast as the older plants were removed every few days new growth replaced them, this continuing until May of 1908. It seems then that bringing the mud containing the oospores into warm laboratory from the winter conditions outside had the same effect as the return of spring upon the plants. This "forcing" process was tried several times since, and though not with such striking results, a good growth of reproductive nanandrous *Œdogonium* was obtained. If the mud is introduced in two large a quantity propor-

tionately to the size of the jar bacterial decomposition will result. The process was also tried with other plants and usually with good results.

Striking results are always obtained with *Vaucheria geminata*. The plant begins to appear late in the fall in its native habitat around springs and in pools. At the beginning of winter it has usually attained a considerable length of the filaments. The winter usually comes on it in the vegetative stage, and in shallow pools it is frozen hard in the ice until Spring. Cakes of ice containing this plant may be put into cold water and within a few hours after melting out be brought into a warm room. Several days later, so great an abundance of zoospores will result that they literally cover the surface of the aquarium, and can be scooped off in all stages of development for study. If the spores are left they will develop into new plants, which in the course of a few weeks will begin to develop an abundance of oogonia and antheridia, while the older plants will disappear. The plant can then in this way be made to produce zoospores any time during the winter season. A rather large aquarium serves best, and flowing or running water is not necessary, nor any regulation of light as is usually prescribed for zoospore production by older methods at other times of the year. *Vaucheria sessilis* will respond as favorably as *Vaucheria geminata* to such treatment but the latter is usually more abundant. *Vaucheria sericea*, a much smaller species than the two preceding has also been used with success.

Diatoms which are usually present in large quantities around springs and in pools and ponds can be taken into the warm laboratory in like manner and within a few hours afterwards will show at their best their characteristic movements. These plants always show their movements best shortly after being brought from colder to warmer water, so that in summer even, their motion are best demonstrated when brought from cold spring water to the warmer surroundings of the laboratory.

By a process of treatment not unlike the "forcing" method just cited, *Hydrodictyon* was last winter made to develop in the laboratory when there was freezing weather outside with several feet of snow on the ground. A jar in which a small amount of this plant was put for preservation during the fall and winter, was put on a window shelf just over a radiator where the heat was excessive and constantly equal day and night. The great heat brought about a

condition of environment not unlike that when this plant is reproducing in July, and with the same results. Nets were produced in all stages and sizes during the rest of the winter and only disappeared late in Spring. In another window and in another room similar results were obtained with the accompanyment of zoospore formation. These zoospores were found in vast numbers escaping through a small opening in the mother cell wall. Specimens of the net in various stages of formation were sent to a student in another university, who induced zoospore formation in the cells by sudden lowering of the temperature and subsequently raising it. Such results seem to me worthy of note as I had never before had occasion to have *growing Hydrodictyon* during the winter months.

To illustrate the regularity with which some algae will reappear in the jars even after all trace of them has been lost, Rivularia may be quoted as an example. Four years ago a good crop of this plant appeared in great abundance in one of the jars early in Spring. It has reappeared every year since then about the same time. *Tolypothrix* has also come out annually in one of the jars the contents of which were not disturbed during several years.

Monoecious forms of *Œdogonium* appear annually and usually early in spring. The first appearance of this plant is usually accompanied with the formation of asexual zoospores which seem to multiply the individual filaments. This stage is usually recognized by the kinky twisted appearance of the plant. The zoospores either escape from small holes formed about the middle of the filaments, or by breaking up the cells usually near one end of each cell in the chain thus causing a zigzag appearance of the filament seen under the microscope, and a kinky interwoven appearance as they can be seen by the naked eye near the surface of the water. Later on as the filaments increase in number, oogonia and antheridia are formed.

Coleochaete scutata is ordinarily considered as a comparatively rare plant. Its periodical appearance in undisturbed aquaria is worthy of note. The plant remains attached to the walls of the aquaria for many months at a time, constantly increasing in diameter, often while the interior cells near the centre have completely rotted away, so that the plant looks like a ring rather than a plate. Such old plants in the laboratory nearly always eventually illustrate a peculiar method of vegetative multiplication. At the edge or near the last ring of cells, there are developed at intervals new centres of

growth, some cell or group of cells acquiring the power of multiplying in all directions and becoming ultimately new plants. Six to ten new plants are often produced from a large old one.

This power of multiplication in radial direction not infrequently is acquired by cells five or six layers inward from the circumference though usually the cells on the perimeter alone become centers for the formation of new plants.

We have found that *Coleochaete scutata*, at least in the vegetative stage is not at all a rare plant, for there is hardly a single month in the year that one or other of the jars does not contain this plant in one or the other stages of development. Dr. G. S. West of the University of Birmingham has written me that though in vegetative stage it is common in England he has never seen it in fruit growing in the laboratory. Plants with antheridia and antherozoids we have had frequently, and nearly every culture shows these when the plants are young. Plants with divided carpospores I have found in the laboratory several years ago two or three times, and fixed material is still on hand.

Desmids in conjugation have never been obtained by us in laboratory cultures, but such cultures were reported to me from the University of California. Vegetative *Closterium* is easily transferred to the laboratory, and there kept for a long time, continually dividing. Conjugating forms of small Spirogyra will appear usually in spring, as also such stages of *Zygnema leiosperma*. Larger jars of ten or more gallons capacity seem favorable to their growth. One to two gallon jars will often produce zygosporic material in spring when well exposed to sunshine. I have found that it is at times only necessary to transfer *Draparnaldia* in early spring from its native habitat in ditches to the laboratory in shallow vessels, in which some organic matter such as bits of meat or flies are put when an abundance of zoospores will be produced. *Chaetophora* too will produce zoospores in the laboratory. Early in summer is also the best time to obtain sexual stages of *Achlya*, or fly-mould. This thrown into stagnant water from ponds first produce oogonia and antheridia and later zoospores exclusively.

Though the water in aquaria where algae grow should never be completely changed at any time it is necessary to replace the water that disappears by evaporation. The plants will of course after a certain time remove all the salts that are present and serve as plant food. These salts can be supplied in two ways. Water is

added from time to time to supply what has evaporated but the amount added at any one time should never exceed one fifth of the total contents of the jar, or the plants may be either killed or seriously retarded in their growth. The water added contains enough salts in solution to last the growing plants for some time. Another method of replacing food removed from the jars in the process of growth is to add the salts directly in the proper proportions as shown by Sachs. Such plant food can be obtained in the form of tablets* and one can be added to a jar every few months. For algae one tablet to every quart of water contents of jar added occasionally gives good results, and is a safer method than adding fresh water, the shock of which often proves fatal. In supplying water removed by evaporation, care must be taken that the water when taken from taps be free from rust, and that accordingly the the water be only taken after it has run for some time. Iron is fatal to Conjugatae. It has been found that it is dangerous or destructive to such a hardy plant as *Marsilia vestita*, one of the Pteridophyta.

A method of obtaining or rather preserving algae alive depends on a principle just opposite to that of "forcing." A considerable number of typical algal forms appear in summer or autumn when they cannot be used for demonstration. Among these are *Hydrodictyon* and *Spirogyra nitida*. By putting these late fruiting plants in a jar in a normal diffusive light in a cool place they will last for months with little change. Spirogyra nitida I have thus kept in fruiting condition till spring, and this plant usually starts zygospore formation at the end of September. Hydrodictyon plants have also been kept in a cool well lighted place without decay until Spring. The reproductive stages at maturity remain a long time unchanged, and are not so subject to decay as vegetative stages. It might not be an entirely unprovitable procedure to attempt the arrest of the growth of plants in desirable stages until needed for demonstration. It might be of interest to find to what extent living plants will submit to preservation in mild state of cold storage until needed for class work.

In regard to collecting algae from the field for class use best results are usually obtained in Spring. Most of the useful plants for class study appear then in our latitude but many remain or re-

* These tablets can be obtained at very reasonable terms from Mr. E. F. Bigelow, Arcadia, Sound Beach, Ct.

appear in various places throughout the summer. Others never come till summer. Small pools and especially such as are dried up part of the year have been found to be most fruitful of the best forms as well as the greatest variety of forms. In large ponds and in streams of some size, or even backwaters of rivers water animals, crustacea, minnows destroy algae. Places even small that are permanently wet may have an abundance of material such as, *Spirogyra*, *Mougeotia*, *Œdogonium*, desmids free floating or as plankton, but oftener plants illustrating reproductive stages will be obtained from small bodies of water, streams or pools where the water has disappeared in summer or fall. Unicellular plants of Protococcoideae are usually found together. *Euglena*, *Pandorina* *Eudorina*, *Gonium* colonies are commonly found in summer as constituents of the green scum on the wallows in fields where pigs are kept. Wet places near troughs where cattle are watered are often covered with *Euglena viridis*. Water pools near barnyards when manure is present also contains *Euglena* and other Protococcoid forms, especially when exposed to the hot sun. The *Pandorina* is usually found showing all stages of formatión of smaller colonies within the old cells.

Pediastrum is usually found growing with Hydrodictyon and can be best obtained from the deposit falling from the floating plants to the bottom of the vessel in which they are kept. Often Pediastrum simplex is found with other unicellular forms or in common plankton floating on ponds. Desmids that secrete mucous are always found in shallow pools or sluggish streams either at the bottom when the water is cold, or rising to the surface when the water is warm and of lower specific gravity. Whenever such plankton is found in abundance which is rarely, conjugating forms will be present. The appearance of the plankton is that of a slimy mass often mucilaginous, but seldom or hardly ever stringy. *Haematococcus* grows only in rain water, and can usually be found in basins or other places where such water collects. It is ordinarily found only after rain.

In collecting as well as growing algae great patience and persistence is necessary. It is always difficult to find the good stages of algae especially the rarer ones even when one has the best equipment for collecting such. The microscope we have found most suitable is an instrument already described in these pages.* One

* MIDLAND NATURALIST, Vol. I, No. 2, p. 53.

must make it a rule to examine every bit of material one meets on a collecting expedition, and it often happens that the best plants are found where they are the least expected. It is a mistake to think that the greenest and best looking scums contain the best plants. Reproductive stages of plants are usually found in yellowish or muddy uninviting masses, whereas the green masses are common vegetative states of algae.

The most important principle of all to follow is, however, that better results and more success will invariably be had rather by doing intensive and thorough work over a very small area, than a superficial and occasional examination of a large tract of country. From the data given above one may get a fairly good idea of what constitutes a likely place where algae are to be found. Once such a locality is fixed it is necessary often to make trips to it, every few days to watch the stages of the various plants present. About three fourths of the useful and rare algae I have found in the last five years were obtained from ditches along a railroad within a quarter of a mile of its length. The collector will soon find in his neighborhood the best place where these plauts are found. By visiting such a place frequently for only a single season, one will eventually be surprised at the success attained. One will soon be able to tell almost at a glance the common forms of cryptogams without microscopical examination, and even the stages of development in which they are. It must, however, be remembered that experience fraught with many a failure is the best instructor, but also that a few remarkable successes in obtaining rare algae will make the collector and grower of these plants feel more than repaid for his efforts.

Birds Found in St. Joseph Co., Ind., Each Day in June, 1909.

BROTHER ALPHONSUS, C. S. C.

As May is the month when most of the birds migrate northward, so June is the nesting time for most of them. And as more species are seen during their migrations than may be found in any one locality when the nesting season begins, the total number of species seen will be considerably smaller in June than in May.